Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2025.334

RESEARCH ARTICLE

Tibetan Herbal Formulations Enhanced the Antioxidant Capacity of Weaned Female Yaks by Modulating the Gut Microbiota

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ARTICLE HISTORY (25-930)

Received:	September 27, 2025
Revised:	December 18, 2025
Accepted:	December 21, 2025
Published online:	December 24, 2025

Key words:
Antioxidant capacity
Gut microbiota
Herbal formulation
Microbial diversity
Weaned yak

ABSTRACT

Tibetan herbal formulations, rooted in traditional medicine, offer natural interventions to enhance livestock health. This study investigated the effects of three Tibetan herbal compound formulations on the gut microbiota of weaned female yaks, which frequently experience health challenges at high altitude. A total of 48 yaks were assigned to four groups (n=12), receiving either one of three herbal-supplemented diets (WYA, WYB, WYC) or unsupplemented fodder (WYD, control) for 30 days. Serum antioxidant parameters including total antioxidant capacity (T-AOC), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and malondialdehyde (MDA) were measured using commercial assay kits, and statistical analysis was performed using one-way ANOVA. Yaks fed diets supplemented with Tibetan herbal formulations exhibited higher T-AOC, SOD, GSH-Px activities and lower MDA levels as compared to the control group (WYD). High-throughput 16S rRNA sequencing revealed significant shifts in microbial diversity and composition. Beta diversity analyses indicated distinct microbial structures in treated groups, with enriched genera such as *Akkermansia, Clostridium*, and *Ruminococcus* in WYA and WYB, potentially enhancing gut barrier function and nutrient metabolism. Differential species analysis (Venn diagrams, heatmaps, t-tests, LDA) further confirmed group-specific taxonomic variation at phylum and genus levels. The improvement in antioxidant capacity corresponded with reduced MDA concentration in supplemented groups. These findings demonstrate that Tibetan herbal formulations dynamically modulate gut microbiota, offering a scientific basis for their use in improving yak health, resilience, and sustainable husbandry practices in the Qinghai-Tibet Plateau.

To Cite This Article: Cidan Y, Cisang Z, Tian J, Zhang X, Safdar M, Pubu P, Zhu Y and Basang W, 2025. Tibetan herbal formulations enhanced the antioxidant capacity of weaned female yaks by modulating the gut microbiota. Pak Vet J, 45(4): 1686-1697. http://dx.doi.org/10.29261/pakvetj/2025.334

INTRODUCTION

Yaks (*Bos grunniens*), indigenous to the Qinghai-Tibet Plateau and adjacent regions of China, exhibit remarkable adaptations to extreme environmental conditions, including cold, hypoxia, and aridity (Yang *et al.*, 2020). In particular, this animal provides meat, milk, and hides for enhancing high-altitude pastoral livelihoods (Zhang *et al.*, 2021; Gao *et al.*, 2020). Even so, life at this altitude still has its own set of limits. The short growing season and low oxygen restrict both forage quality and animal performance, and productivity rarely meets the needs of local farmers.

Compared with other livestock industries in China, yak farming has progressed slowly. The sector remains tied to open-range grazing and minimal technological input (Li *et al.*, 2023). As a result, reproductive rates are low and disease problems remain common. Calf diarrhea, slow growth, and weak immune function in weaned females continue to cause major economic loss (Bernal-

Córdoba *et al.*, 2022). For many producers, improving health and breeding efficiency has become more urgent than expanding the herd size.

Tibetan herbal formulations, derived from traditional medicinal practices, offer a promising, low-risk approach to improve livestock health (Yang *et al.*, 2023). Some of these herbs like *Coptis (C.) chinensis* and *Pulsatilla (P.) chinensis* exert antibacterial and anti-inflammatory effects (Li *et al.*, 2023; Liu *et al.*, 2023), *Prunus (Pr.) mume* and *Terminalia (T.) chebula* provide astringent and antidiarrheal benefits (Kong *et al.*, 2014; Tian *et al.*, 2023; Huan *et al.*, 2024), *Rheum (R.) palmatum* and *Plantago (Pl.) asiatica* contribute heat-clearing and detoxifying functions and *Glycyrrhiza (G.) uralensis* harmonizes the formulation (Li *et al.*, 2019). These interventions target gut microbiota modulation to mitigate health challenges and enhance resilience.

During the past decade, improvements in sequencing and bioinformatic analysis have made it possible to examine the yak gut microbiome in detail (Clarridge, 2004; Wang *et al.*, 2022; Xu *et al.*, 2025). Even so, there is still limited information about how multi-herbal Tibetan formulations affect gut microbiota or antioxidant responses in female weaned yaks raised under high-altitude stress. In this study, we explored whether dietary supplementation with three herbal combinations could enhance antioxidant status through shifts in beneficial gut microbiota. The findings are expected to provide experimental support for integrating traditional Tibetan herbal knowledge into modern yak production systems across the plateau.

MATERIALS AND METHODS

Ethical approval statement: All experimental procedures were conducted under the supervision and approval of the Animal Welfare and Ethics Committee of the Institute of Animal Husbandry and Veterinary Medicine, Xizang Academy of Agriculture and Animal Husbandry Science, and were in accordance with the ethical guidelines of Nanjing Agricultural University (Approval No. NJAU-20220305025). The research was carried out in collaboration with Linzhou Jingtu Company, ensuring compliance with institutional and national standards for animal experimentation.

Experimental design: A total of 48 weaned female yaks were selected from a yak farm in Tibet, China and randomly divided into four groups, with each group consisting of 12 yaks. Each group received a distinct diet for 30 days. The WYA group was fed Tibetan herbal compound formula 1, comprising 40g *C. chinensis* (Huang Lian), 30g *P. chinensis* (Bai Tou Weng), 30g *Pr. mume* (Wu Mei), 30g *T. chebula* (He Zi), 30g *R. palmatum* (Xizang Da Huang), 30g *Pl. asiatica* (Che Qian Zi), and 30g *G. uralensis* (Gan Cao). The WYB group received Tibetan herbal compound formula 2, consisting of 60g *P. chinensis* (Bai Tou Weng), 30g *C. chinensis* (Huang Lian), 30g *Artemisia anomala* (A Shi Hao), 30g *Saussurea lappa* (Zang Mu Xiang), 30g *Paeonia lactiflora* (Bai Shao), 30g *Hyoscyamus niger* (Shan Lang Dang), and 30g *G. uralensis* (Gan Cao). The WYC group was provided with Tibetan herbal compound formula 3, containing 30g *Taraxacum mongolicum* (Pu Gong Ying), 25g *T. chebula* (He Zi), 15g *Hyoscyamus niger* (Shan Lang Dang), 15g *Magnolia officinalis* (Hou Po), and 15g *Pueraria lobata* (Ge Gen). The WYD group, serving as the control, was fed standard fodder without any Tibetan herbal compound formula. The herbal compounds were thoroughly mixed into the daily fodder, and all yaks had unlimited access to water and standard fodder throughout the experiment. Fecal and blood samples were collected on days 15 and 30 of the feeding period.

Determination of serum antioxidant indicators: Serum antioxidant indicators were measured by determining glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), total antioxidant capacity (T-AOC), and malondialdehyde (MDA) using kits from Nanjing Jiancheng Biotechnology Research Institute. All experiments were done in triplicate for reproducibility.

DNA extraction and 16S rRNA gene amplification: From 48 yaks, six fecal samples per group (total n=24) were randomly selected for sequencing to represent microbial diversity. Therefore, microbial DNA was extracted from 24 samples using the Soil DNA Extraction Kit. The DNA concentration and purity were determined using a NanoDrop NC2000 spectrophotometer, and DNA integrity was verified by 1.2% agarose gel electrophoresis. The V3-V4 hypervariable region of the bacterial 16S rRNA gene was amplified by PCR using specific primers. PCR products were purified using Vazyme VAHTSTM DNA Clean Beads (Vazyme, Nanjing, China). Amplified products were quantified fluorometrically with the Quant-iT PicoGreen dsDNA Assay Kit on a BioTek FLx800 Microplate Reader. Based on fluorescence quantification, samples were pooled in equimolar ratios for sequencing. A sequencing library was constructed using the TruSeq Nano DNA LT Library Prep Kit (Illumina, San Diego, CA, USA). Library quality was assessed using an Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA) and a Promega QuantiFluor system (Promega Corporation, Madison, WI, USA) before sequencing on an Illumina platform.

Bioinformatics analysis: Microbiome data were processed using QIIME 2 (version 2019.4). Raw data were demultiplexed, and primers were removed. Quality filtering, denoising, sequence merging, and chimera removal were performed to generate non-singleton amplicon sequence variants (ASVs). ASVs were aligned using MAFFT, and a phylogenetic tree was constructed with FastTree2. Alpha and beta diversity metrics were calculated using the QIIME 2 diversity plugin. Taxonomic classification of ASVs was performed with the classify-sklearn naïve Bayes classifier in the feature-classifier plugin. The ASV data was analyzed to determine the microbial community composition at various taxonomic levels (phylum and genus) for each sample done by sequencing, then visualized using R (version 4.2.1). Venn diagrams and heatmaps were constructed in R to investigate microbial community structure differences. Significant differences at the level of taxa between groups were identified using LEfSe and t-tests. Abundances of microbes were compared pairwise using the metagenomeSeq method. Association network analysis was done in R to show interactions among bacterial phyla in gut microbiota.

Statistical analysis: Amplicon sequencing data analyses were done by one-way ANOVA using GraphPad Prism, version 8.0, and SPSS, version 20.0. Final values are given as mean±SD. Statistical significance was defined as P<0.05.

RESULTS

Antioxidant capacity analysis: Antioxidant capacity was assessed in yaks by determining serum T-AOC, SOD, GSH-Px, and MDA level (Fig. 1). Yaks fed Tibetan herbal compound formula-supplemented diets (WYA, WYB, WYC) had higher T-AOC, SOD, and GSH-Px activities than the control group during the 30-day feeding period (P<0.05). Moreover, throughout the experimental period, the levels of MDA, as an indicator of oxidative stress, were relatively lower in the experimental groups compared to the control, suggesting a decrease in lipid peroxidation in the yaks due to herbal formulation feeding.

Sequencing Analysis: Accordingly, a total of 1,856,636 non-singleton ASVs were obtained for the four groups: WYA (423,509 ASVs), WYB (474,605 ASVs), WYC (449,181 ASVs), and WYD (509,341 ASVs) (Table 1). The Shannon diversity index-based rarefaction curves for all the groups reached a plateau, demonstrating that the sequencing depth was enough and that microbial diversity was adequately sampled (Fig. 2).

Microbial community diversity analysis: Seven alpha diversity indices were used in the present study, and for all groups on days 15 and 30, Good's coverage was greater than 99.3%, indicating sufficient sampling of the microbial community. Alpha diversity indices did not show significant differences among groups in box plots (P<0.05; Fig. 3A, B). Beta diversity was determined by PCoA and represented clear separation of the WYD group from the WYA, WYB, and WYC groups, indicating that Tibetan herbal compound formulas modified the gut microbial structure of yaks (Fig. 3C, D).

Gut Bacterial Composition analysis: At the phylum level, the dominant populations in samples from days 15 and 30 were Firmicutes, Bacteroidetes, Verrucomicrobia, and Actinobacteria, together accounting for about 95.9% of the bacterial community across all groups (Fig. 4A, B). At the genus level, on day 15, Arthrobacter was the dominant genus in both WYB and WYD groups, with Akkermansia and 5-7N15. In the WYA group, *Akkermansia* predominated, followed by *5-7N15* and *Oscillospira*. In the WYC group, *Akkermansia* was the most abundant, followed by *Arthrobacter* and *5-7N15* (Fig. 4C, E). On day 30, *Arthrobacter* and *Akkermansia* remained the top two genera across all groups. In WYA, *Bacillus* was the third most abundant genus, while *Oscillospira* showed higher relative abundance in WYB compared to other groups. In WYC and WYD, *5-7N15* was the third dominant genus (Fig. 4D, F).

Table 1: Bacterial sequencing data summary showing number of non-singleton amplicon sequence variants (ASVs) obtained for each sample across the four groups (WYA, WYB, WYC, WYD)

SampleID	Input	Filtered	Denoised	Merged	Non-chimeric	Non-singleton
WYA1	67948	63720	60318	42026	35807	35694
WYA2	61414	57701	54950	42589	35736	35606
WYA3	63812	60106	56856	39305	33822	33708
WYA4	57255	53717	51119	36062	32195	32085
WYA5	62955	58824	55507	36698	29005	28841
WYA6	59414	55882	53038	36346	31661	31563
WYA7	77685	73276	69225	44704	37222	37046
WYA8	69262	64931	61158	39803	33020	32827
WYA9	76525	72574	68543	44740	37620	37446
WYA10	80026	75034	71102	47274	38947	38712
WYA11	79921	75271	71355	47621	39101	38916
WYA12	80706	76152	72405	50530	41270	41065
WYB1	61328	57861	55374	40895	35544	35477
WYB2	62598	59022	55829	39507	34965	34852
WYB3	79894	75216	72057	51647	44937	44799
WYB4	70158	66096	62672	41756	35059	34810
WYB5	64921	61189	57758	40379	35920	35802
WYB6	61855	57989	55175	39281	33759	33651
WYB7	78601	74103	70406	49316	42085	41972
WYB8	80226	75020	71371	48759	42471	42328
WYB9	85561	80835	76519	50933	43885	43754
WYB10	76311	71899	67760	44245	38644	38509
WYB11	83642	78477	74409	48883	41855	41671
WYB12	85256	79979	76555	55128	47135	46980
WYC1	68774	64714	61743	44121	37916	37821
WYC2	70487	66109	62966	44094	36512	36380
WYC3	64626	61176	58119	41560	34926	34824
WYC4	61982	58041	55223	39280	33491	33404
WYC5	60295	56571	53371	35472	31383	31279
WYC6	67532	64069	60251	39377	33897	33738
WYC7	86343	81419	77587	53925	44940	44801
WYC8	94015	88626	84018	55801	45470	45210
WYC9	83111	78193	74815	55373	47932	47767
WYC10	75890	71258	67487	44571	37486	37343
WYC11	73905	69543	65613	41215	35708	35580
WYC12	64400	60722	57150	36810	31187	31034
WYD1	69374	65300	62051	42164	35943	35813
WYD2	62119	58415	55822	41543	37535	37465
WYD3	62573	58475	55915	41506	32120	32007
WYD4	118296	107651	101796	67566	56630	56352
WYD5	68198	64605	62112	45052	34559	34454
WYD6	66899	62414	59876	46624	39218	39084
WYD7	77896	73388	70214	49919	39827	39709
WYD8	80444	75559	72775	56323	46496	46400
WYD9	76828	72407	69329	50012	38926	38834
WYD10	85049	80390	77543	60135	53163	53118
WYD11	86071	81343	77920	55848	42667	42457
WYD12	100004	93786	90754	71290	53784	53648

Species differential analysis: Venn diagrams illustrated ASV overlap among groups. On day 15, 1,804 ASVs were shared across all four groups, while on day 30, 1,674 ASVs were common (Fig. 5A, B). Heatmaps of genus-level species composition highlighted differences in microbial abundance across samples (Fig. 5C, D). LEfSe and t-tests showed significantly enriched taxa in this study.

On day 15, *Verrucomicrobia* (phylum) and *Clostridium*, *Ruminococcus*, and *Akkermansia* (genera) were enriched in WYA; *Clostridium* and *Arthrobacter* were enriched in WYB; and *Bacillus* was enriched in WYD. No significantly enriched taxa were identified in WYC (Fig. 5E, F). On day 30, significant differences were observed in *Bacteroidales* (between WYA and WYB), *Clostridiales* (between WYC and WYA, and WYB and WYD), and *Bacteroidales* (between WYC and WYD). The WYB group showed more enriched core microbes compared to WYD (Fig. 6A-E).

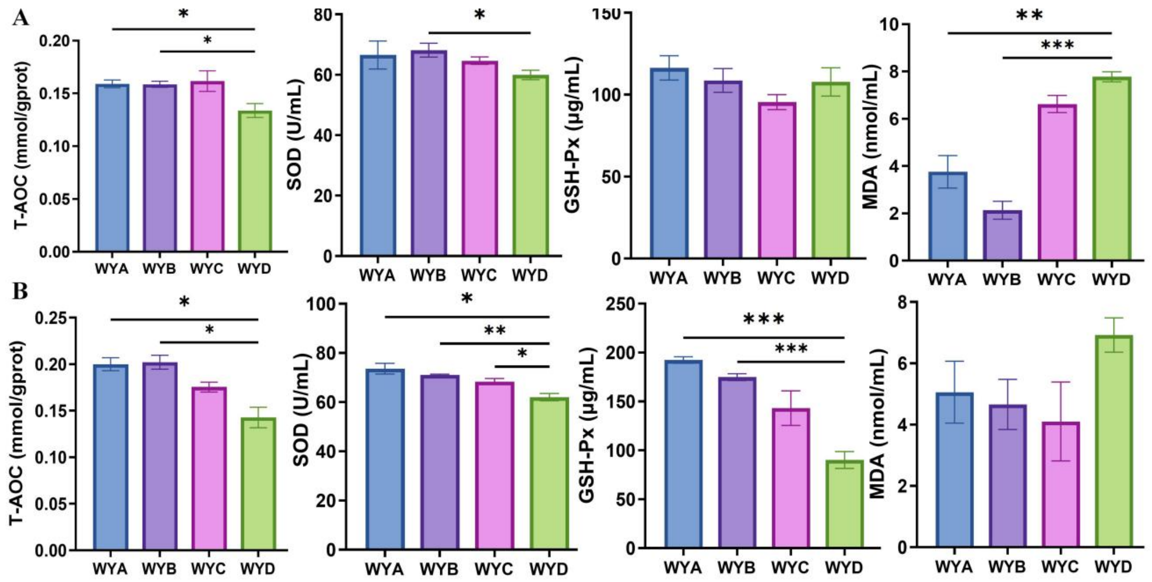

Fig. 1: Effects of tibetan herbal formulas on antioxidant capacity in weaned female Yaks: Serum antioxidant parameters, including total antioxidant capacity (T-AOC), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and malondialdehyde (MDA), were measured in yaks fed different Tibetan herbal compound formulas. (A) Data from samples collected on day 15 of the experiment. (B) Data from samples collected on day 30 of the experiment. Data are presented as mean ± SEM. Statistical significance is denoted as $P<0.05$, * $P<0.05$, ** $P<0.05$.

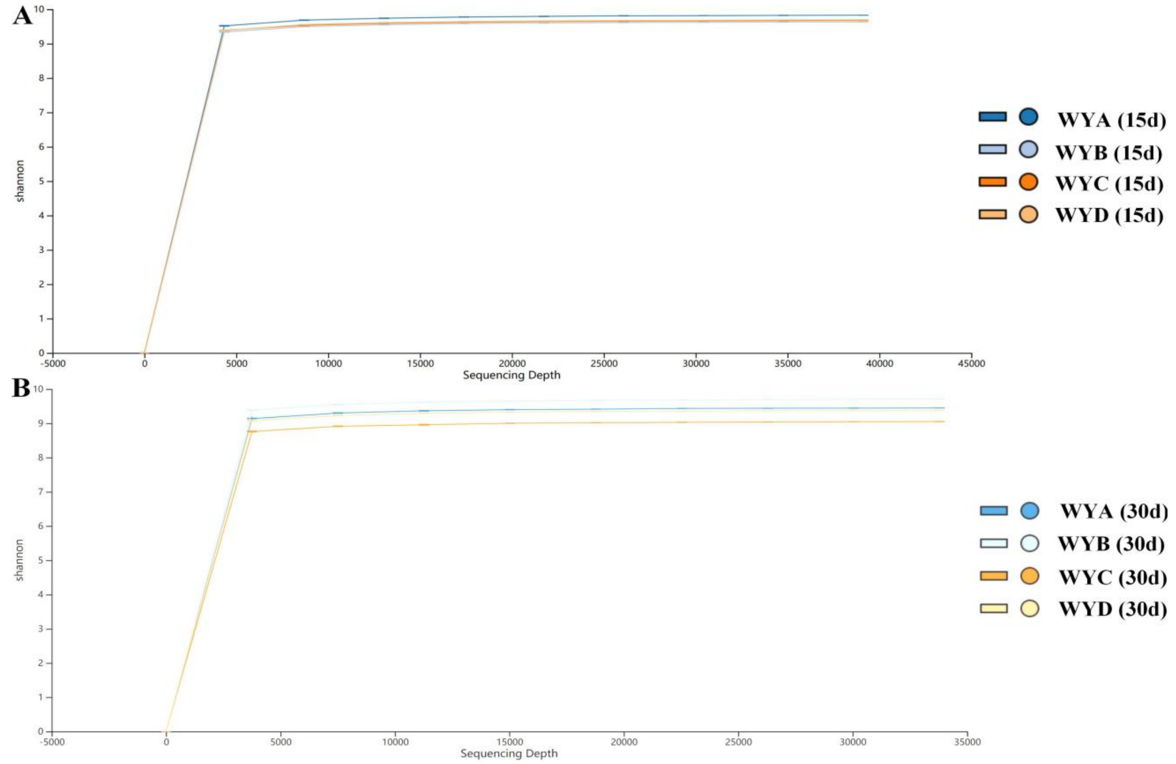

Fig. 2: Rarefaction curves of microbial diversity: Rarefaction curves based on the Shannon diversity index for the four experimental groups (WYA, WYB, WYC, WYD). (A) Curves for samples collected on day 15. (B) Curves for samples collected on day 30. The plateauing of curves indicates sufficient sequencing depth to capture microbial diversity.

Furthermore, LEfSe and t-test analyses comparing experimental groups to the control revealed specific enrichments: on day 15, *Lachnospiraceae* (family) was enriched in WYA, *UBA6857* (genus) in WYB, and *SFMI01* (genus) in WYD (Fig. 7A-C). On day 30, *Lenti01* (genus) and *Cryptobacteroides* (genus) were enriched in WYD compared to WYA and WYB, respectively, while WYC showed no unique enrichments compared to day 15. In WYD, *Lenti01*, *UBA3857*, and *RUG472* (genera) were enriched (Figure 7D-F). T-test analysis identified enriched phyla on day 15, with *Firmicutes* and *Tenericutes* more abundant in WYA ($P<0.05$; Fig. 8A). No phylum-level differences were detected on day 30.

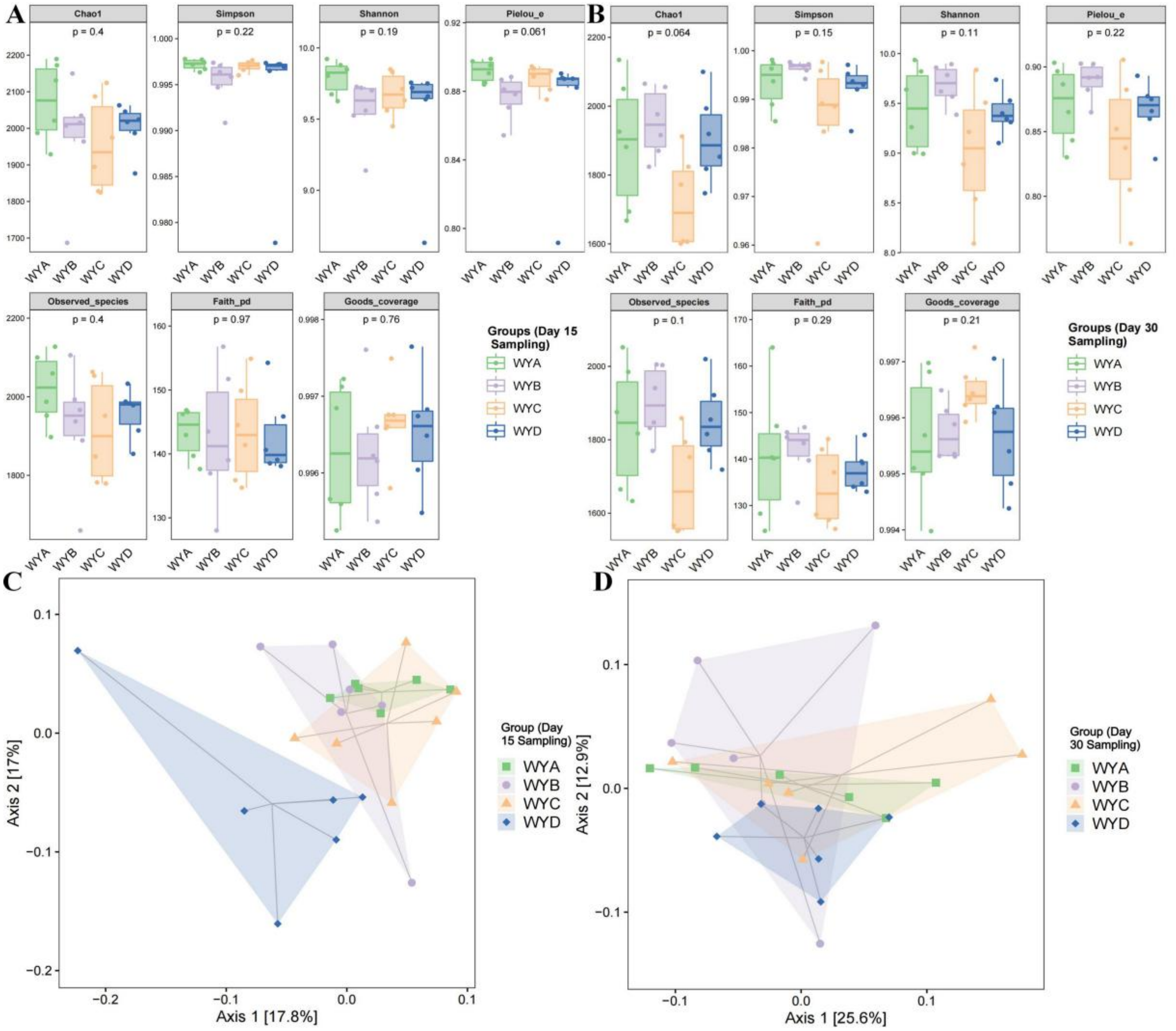

Fig. 3: Alpha and beta diversity of gut microbiota: (A) Alpha diversity indices for the four groups based on samples collected on day 15. (B) Alpha diversity indices for samples collected on day 30. (C) Principal coordinates analysis (PCoA) based on weighted UniFrac distance for samples collected on day 15. (D) PCoA based on weighted UniFrac distance for samples collected on day 30. PCoA plots illustrate differences in microbial community structure among groups.

At the genus level, *Bacillus* was enriched in WYD, while *Adlercreutzia*, *Blautia*, *Streptococcus*, and *Shuttleworthia* were enriched in experimental groups on day 15. Compared to WYD, WYA had higher abundances of *Oscillospira*, *Ruminococcus*, and *Coprococcus*; WYB had more *Eubacterium* and *Thiobacillus*; and WYC had enriched *Clostridium* and *Anaerostipes* (Fig. 8B). On day 30, *Adlercreutzia* was enriched in WYA, while *Atopobium* and *Prevotella* were less abundant. *Butyrivibrio* and *L7A_E11* were enriched in WYB, and *Sporosarcina* was enriched in WYC, while *BF311* and *Veillonella* were less abundant (Fig. 9).

Correlation network analysis: Using the R language package, an association network analysis graph was created to illustrate the relationships between various bacterial phyla in the gut microbiota (Fig. 6F). Nodes were colored to indicate phylum-level taxonomic information, and the links between nodes symbolized correlations, with red lines highlighting positive correlations and green lines signifying negative correlations.

DISCUSSION

Traditional Chinese medicine (TCM) has been widely utilized in veterinary practice to prevent and treat various animal diseases, including respiratory conditions in poultry, gastrointestinal disorders in pigs, and mastitis in cattle (Sawuer *et al.*, 2022; Sankararaman *et al.*, 2022). The present study investigated the changes of the gut microbiota in weaned female yaks following 30 days of supplementation with three different Tibetan herbal compound formulas, sampling at days 15 and 30 to capture temporal dynamics. The design allows for the investigation of additive or time-specific effects of the herbal formulas on the gut microbiome and furthermore allows for insights into temporal changes of microbial communities and their potential implications for health in yaks.

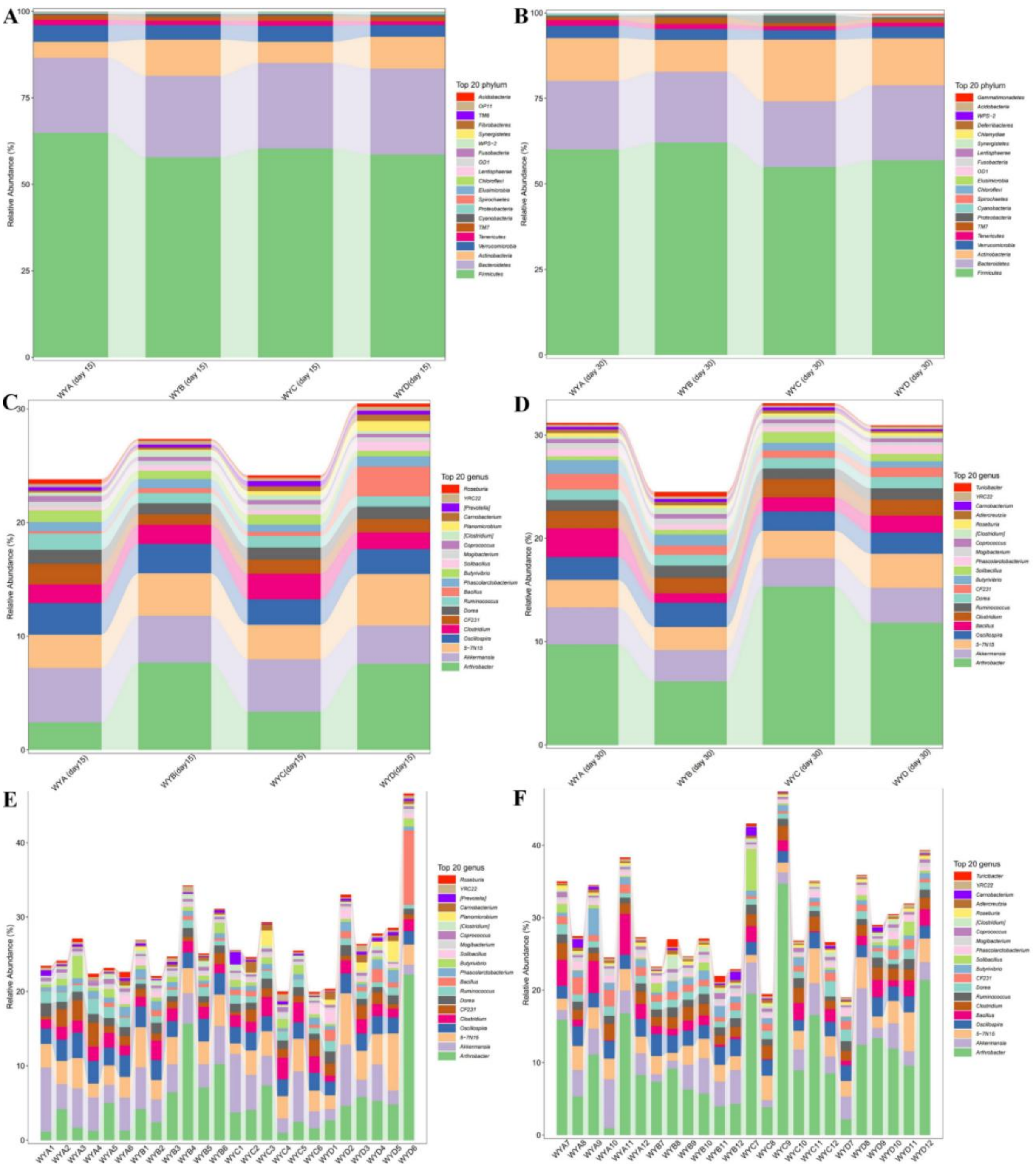

Fig. 4: Relative abundance of gut microbial taxa: Bar charts depicting the composition of gut microbiota in the four groups. (A, B) Relative abundance at the phylum level for samples collected on days 15 and 30, respectively. (C, D) Relative abundance at the genus level for samples collected on days 15 and 30, respectively. (E, F) Relative abundance at the genus level for individual samples collected on days 15 and 30, respectively.

Alpha diversity indices did not differ between treatments (WYA, WYB, WYC, and control WYD), indicating that there was no impact of the herbal formulations on total microbial richness or evenness. Beta diversity analysis by PCoA, however, showed that the overall microbial community structure in the herbal-treated groups was different from that in the control, and the overall composition of the gut microbiota shifted over time in response to the three different Tibetan herbal formulas. In fact, these findings emphasize the dynamic influence of traditional Chinese medicine on gut microbiota in post-weaning yaks in good agreement with the modulatory roles of herbal compounds in gut ecosystems.

At the phylum level, Firmicutes, Bacteroidetes, Verrucomicrobia, and Actinobacteria were dominant in all the groups on days 15 and 30, comprising about 95.9% of the bacterial community, which is consistent with previous studies on ruminant gut microbiomes (Aricha *et al.*, 2021; Langille *et al.*, 2013). Firmicutes have been reported to produce B-group vitamins and exert anti-inflammatory properties that may help improve intestinal

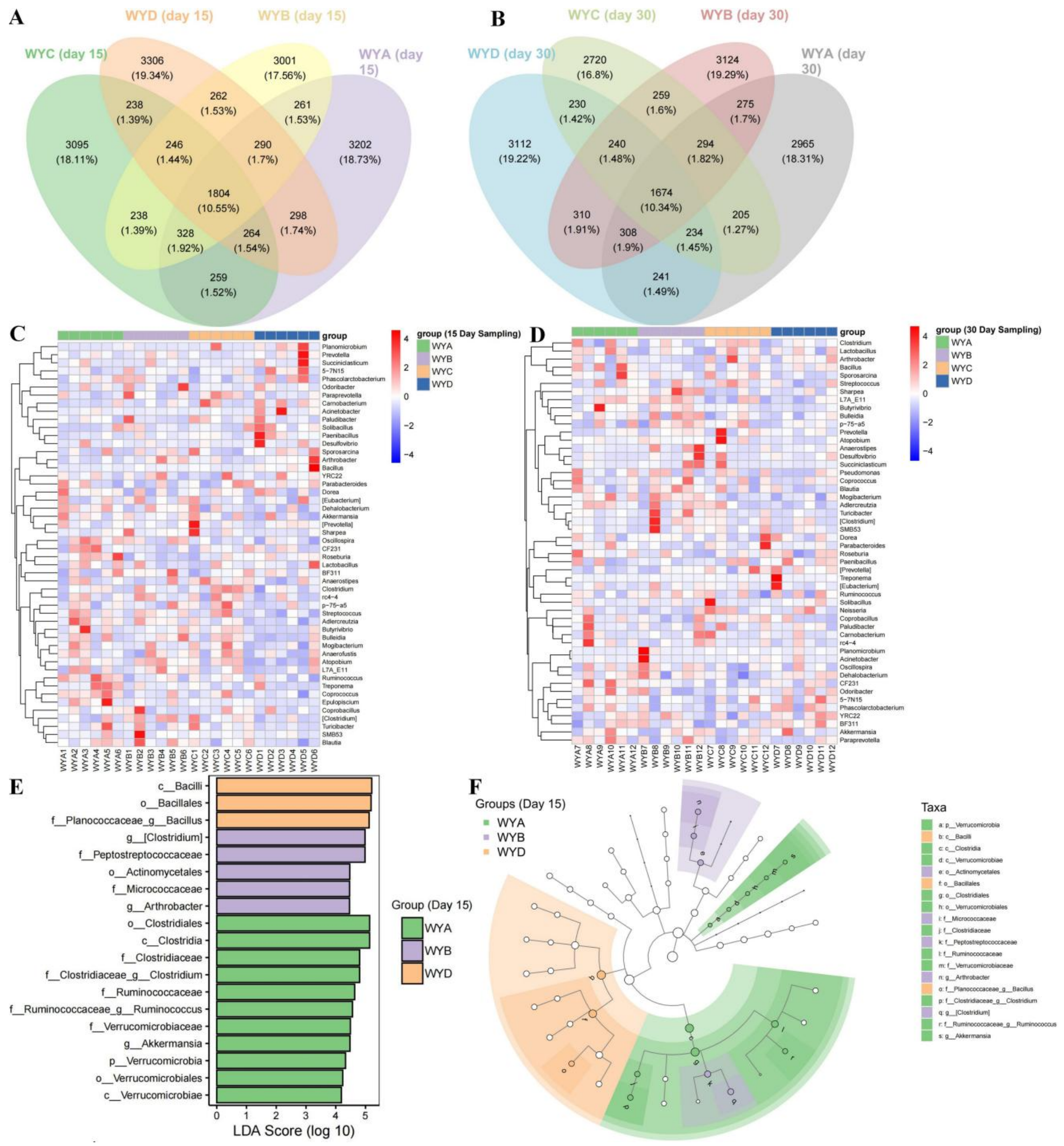

Fig. 5: Microbial community overlap and differential analysis: (A, B) Venn diagrams showing shared and unique amplicon sequence variants (ASVs) among the four groups for samples collected on days 15 and 30, respectively. (C, D) Heatmaps illustrating genus-level microbial community clustering for samples collected on days 15 and 30, respectively. (E, F) Linear discriminant analysis effect size (LEfSe) results, showing taxonomic cladograms and LDA scores (>4) for significantly enriched taxa in samples collected on days 15 and 30, respectively.

barrier function (Kaur *et al.*, 2019). Bacteroidetes have the capability of degrading dietary polysaccharides and synthesizing essential vitamins and coenzymes playing a vital role in metabolic processes (Magne *et al.*, 2020). On day 15, Arthrobacter was predominant both in WYB and WYD and Akkermansia in WYA and WYC. Similarly, on day 30, Arthrobacter and Akkermansia were predominant across all groups, with the third most abundant genera being Bacillus (WYA), Oscillospira (WYB), and 5-7N15 (WYC and WYD). Arthrobacter species have been reported to be resilient in stressful environments, and this bacterium may contribute significantly to microbial stability due to the production of various enzymes and bioactive compounds with possible anti-microbial and probiotic properties (Hernández-Fernández *et al.*, 2022; Sutthiwong *et al.*, 2023). Akkermansia, more so Akkermansia muciniphila, plays an important role in intestinal barrier integrity maintenance and exerts anti-inflammatory effects whose low abundance has been associated with inflammatory diseases (Derrien *et al.*, 2017; Zhou *et al.*, 2020; Cani *et al.*, 2022). Thus, this bacterium is usually found to be less abundant in individuals suffering from an inflammatory condition, indicating that its presence is protective. Bacillus spp. improves gut health by suppressing the growth of pathogens, modulating the immune system, and enhancing the growth of beneficial microbes (Bahaddad *et al.*, 2023). Oscillospira has been related to favorable metabolic health, such as lower body mass index and improvements in lipid profiles, suggesting its role in maintaining gut homeostasis (Nagpal *et al.*, 2018; Yang *et al.*, 2021).

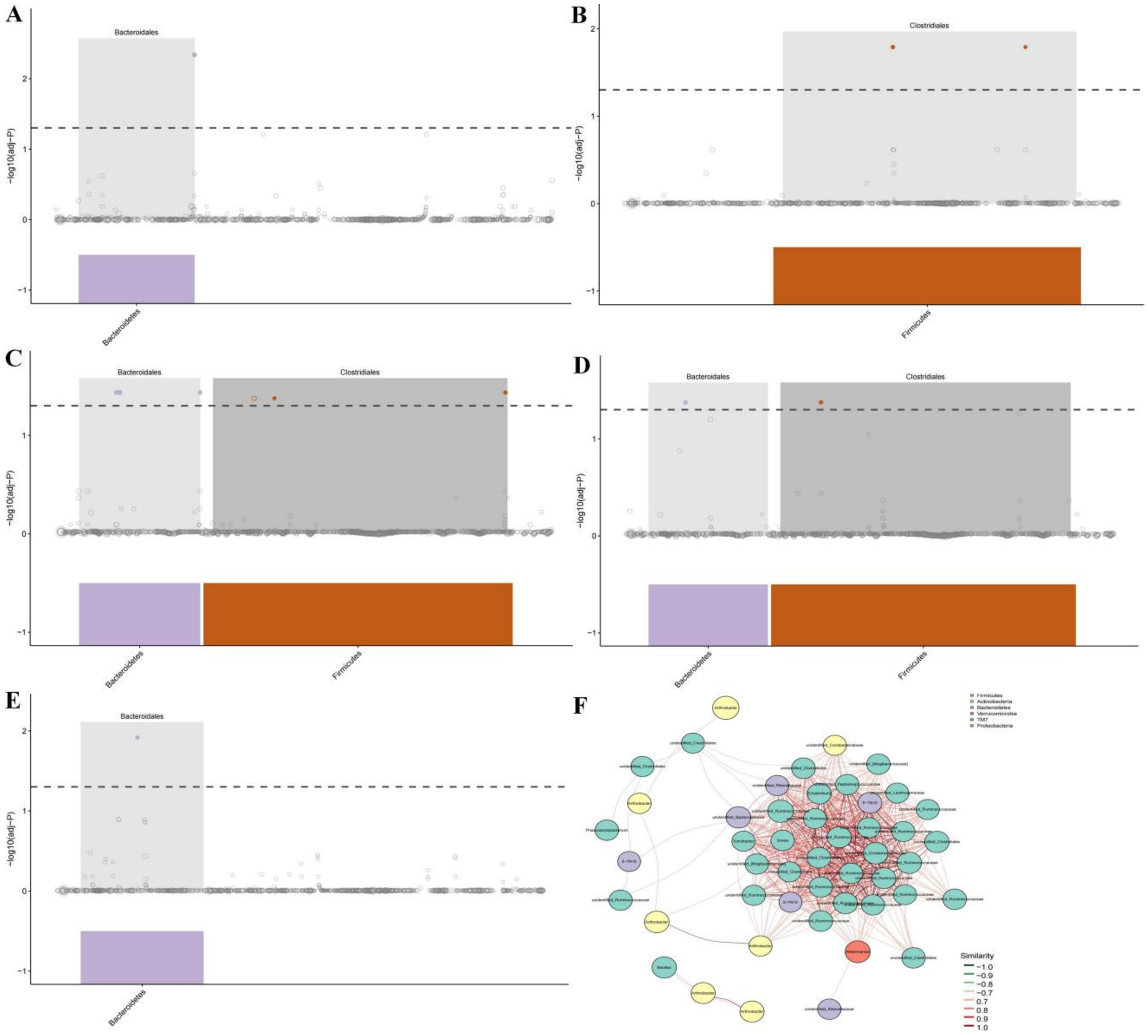

Fig. 6: MetagenomeSeq and correlation network analysis of gut microbiota: MetagenomeSeq analysis of samples collected on day 30, showing significantly enriched core microbes. (A) WYA versus WYB. (B) WYC versus WYA. (C) WYB versus WYD. (D) WYD versus WYB. (E) WYD versus WYC. The x-axis represents ASVs sorted by taxonomic classification (phylum to species), and the y-axis represents -log10(adjusted p-value). Dot size indicates relative abundance (log2(CPM/n); CPM: copies per million; n: sample number). Significant ASVs (above the dashed line) are shown as colored solid dots; non-significant ASVs (below the dashed line) are gray circles. (F) Correlation network analysis of gut microbiota, with nodes colored by phylum and edges indicating positive (red) or negative (green) correlations.

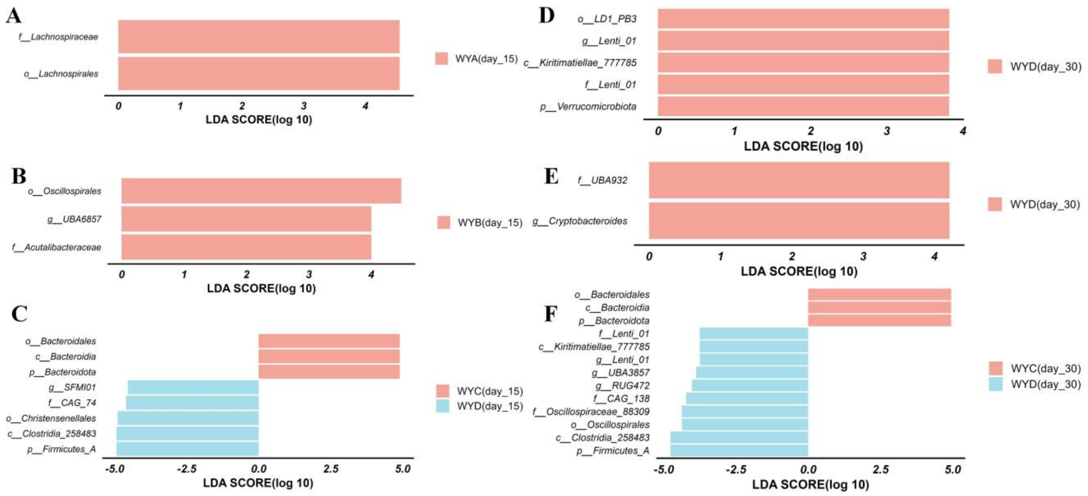

Fig. 7: LEfSe Analysis of gut bacterial flora between experimental and control groups: Linear discriminant analysis (LDA) scores (>4) for significantly enriched taxa. (A) WYA versus WYD (day 15). (B) WYB versus WYD (day 15). (C) WYC versus WYD (day 15). (D) WYA versus WYD (day 30). (E) WYB versus WYD (day 30). (F) WYC versus WYD (day 30).

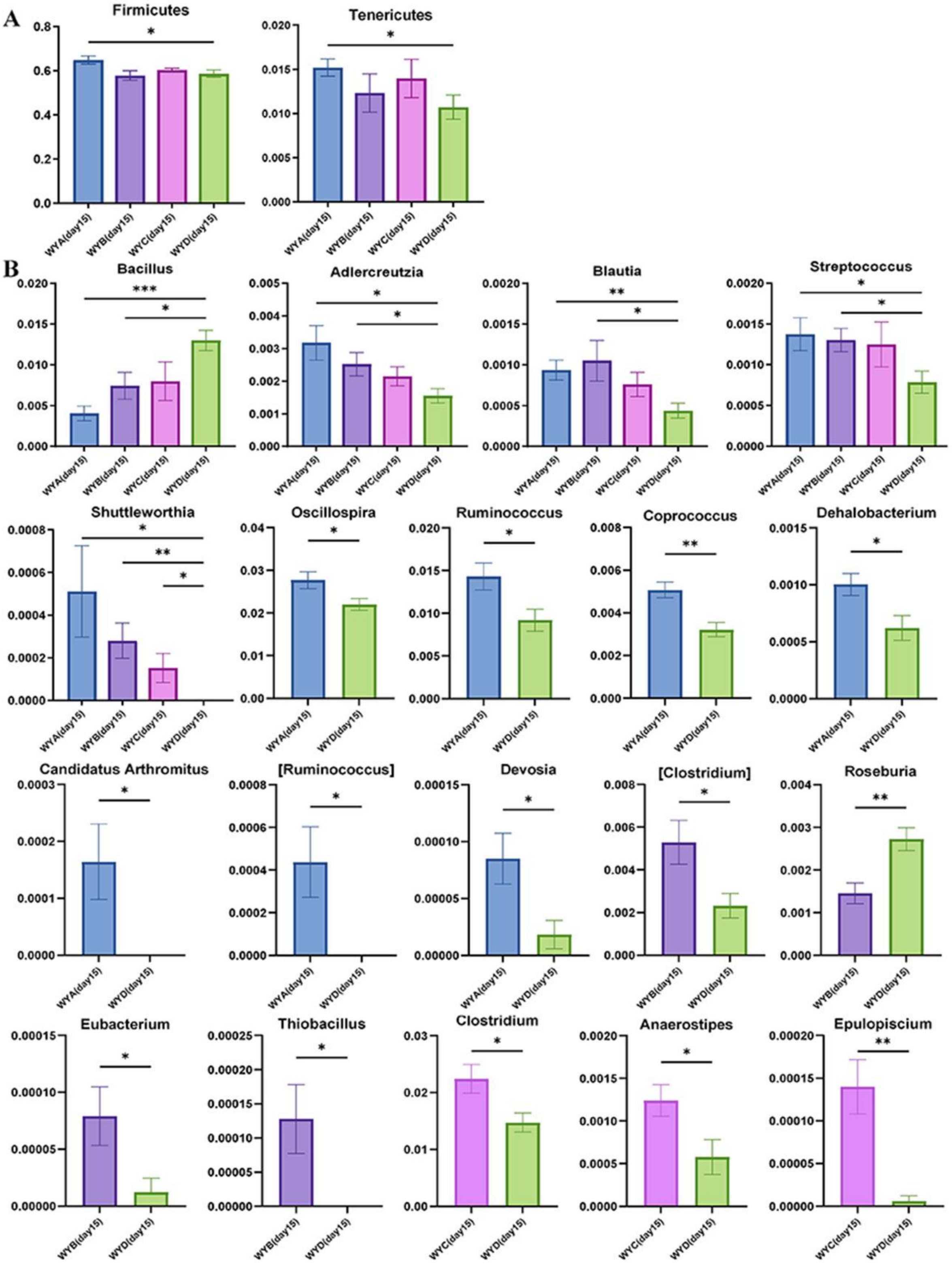

Fig. 8: T-test analysis of differentially abundant taxa on day 15: T-test results showing significant differences in microbial taxa at the phylum and genus levels for samples collected on day 15. Statistical significance is denoted as P<0.05, *P<0.01.

Venn diagram, heatmap, and LEfSe differential analysis showed that herbal treatments induced different microbial shifts. Accordingly, on day 15, significant enrichments were observed in Verrucomicrobia (phylum) and in Clostridium, Ruminococcus, and Akkermansia (genera) for WYA; Clostridium and Arthrobacter for WYB; Bacillus for WYD, while WYC did not show any significant differences, which means that the impact of herbal formula 3 was minimum at this time point. Clostridium spp. could produce short-chain fatty acids

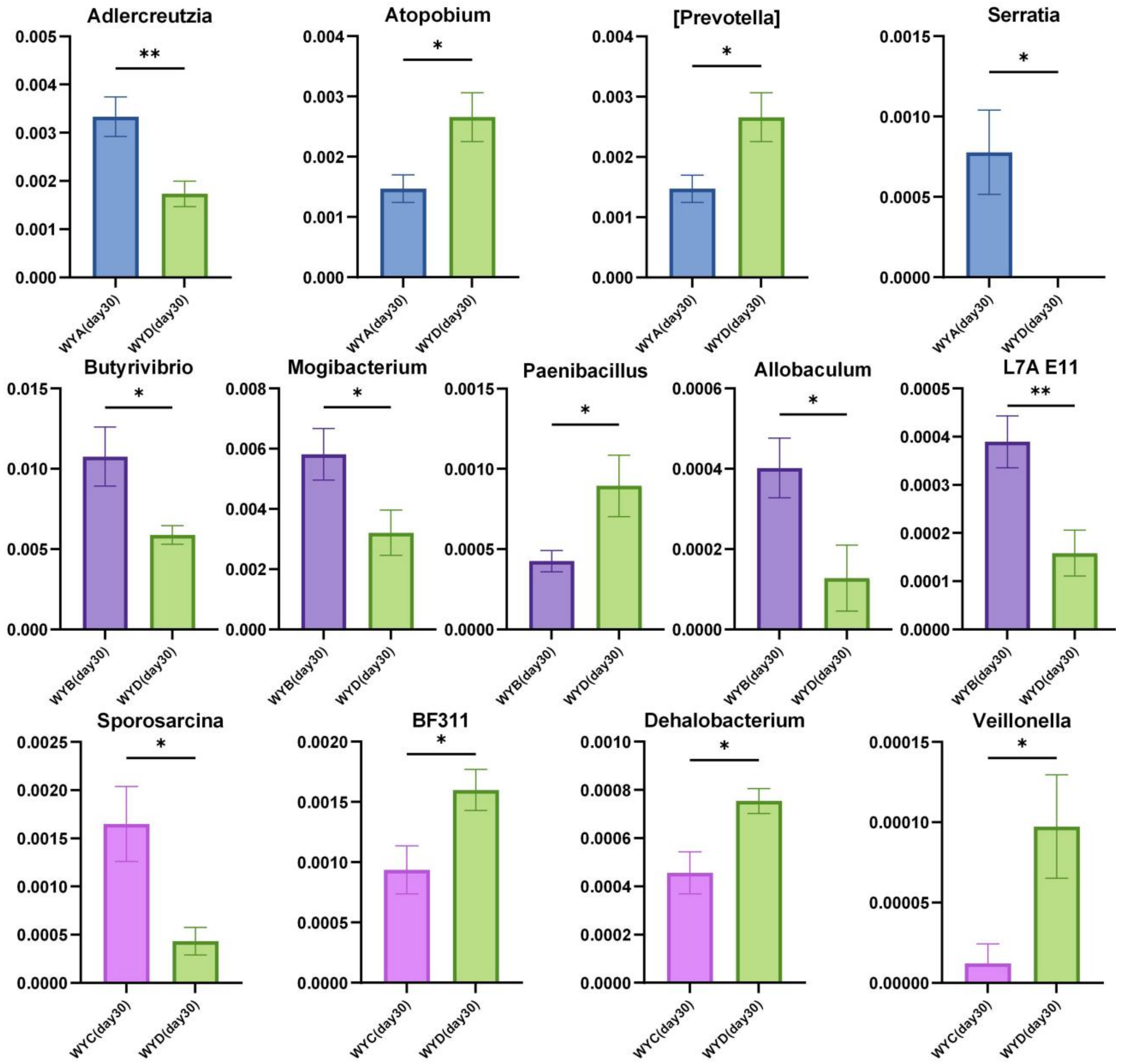

Fig. 9: T-test analysis of differentially abundant genera on day 30: T-test results showing significant differences in microbial genera for samples collected on day 30. Statistical significance is denoted as P<0.05, *P<0.01.

like acetate, propionate, and butyrate that provide nutritional support to intestinal epithelial cells, modulate immune responses, and diminish inflammation (Li *et al.*, 2023). These SCFAs strengthen the intestinal barrier, hence decreasing the possibility of leaky gut and pathogen invasion (Guo *et al.*, 2020). Ruminococcus spp. helps in the degradation of complicated carbohydrates, which improves nutrient absorption (Crost *et al.*, 2016; Devendran *et al.*, 2016). Adlercreutzia, enriched in WYA and WYB, could produce propionate and acetate, while its low abundance has been related to intestinal inflammation (Galipeau *et al.*, 2021; Yang *et al.*, 2024). Notably, Adlercreutzia equolifaciens has anti-inflammatory properties, hence may play a symbiotic role (Oñate *et al.*, 2023). Other increased abundant genera in the experimental group, such as Blautia, Dehalobacterium, and Shuttleworthia, have been reported to have potential probiotic effects (Liu *et al.*, 2021; Miragoli *et al.*, 2021; Chen *et al.*, 2023). All these results indicated that herbal formula 1 and 2 strongly modulated the gut microbiota of weaned yaks through the enrichment of some beneficial taxa at day 15.

By day 30, metagenomeSeq and t-test analyses revealed significant differences in Bacteroidales (between WYA and WYB) and Clostridiales (between WYC and WYA, and WYB and WYD), confirming that these taxa responded well to herbal treatments. The functional contributions of these taxa in microbial community changes must be further investigated. Since the compositions are different, there was a reasonable basis for their distinct effects due to the three herbal formulas. Formulas 1 and 2 contain Coptis chinensis (Huang Lian) and Pulsatilla chinensis (Bai Tou Weng), most likely due to their inhibitory effects against microbes, thus structuring the microbial communities (Zhong *et al.*, 2022; Chen *et al.*, 2023). Moreover, Pulsatilla decoction has been found to induce autophagy by initiating AMPK phosphorylation and suppressing mTOR expression, hence repairing the intestinal epithelial barrier in colitis models (Pan *et al.*, 2024). Although, Formula 3 had limited effects at day 15, it had an obvious effect by day 30, which may mean that it played a delayed or accumulated role in reshaping microbial composition. These changes in microbial diversity could suggest that the Tibetan herbal formulations promote intestinal health by enriching beneficial bacteria and subsequently improving nutrient metabolism and immune function, enhancing resilience to environmental stress in weaned yaks. Temporal dynamics thus reflect immediate and sustained modulation of the gut microbiota, supporting implications for optimisation of yak husbandry.

Conclusions: The results indicate that dietary Tibetan herbal mixtures can substantially reshape the gut microbial landscape of weaned female yaks. Sequencing data highlight concurrent changes in both microbial diversity and composition associated with specific supplementation.Noticeably, genera such as Akkermansia, Clostridium, and Ruminococcus were enriched, suggesting these taxa may contribute to intestinal function stabilization and improvement of nutrient use. Such enrichment of beneficial microbes is associated with better digestive efficiency and/or more balanced immune function. Collectively, these findings provide solid experimental support for the practical application of Tibetan herbal formulations in yak production systems and offer some mechanistic insights into how plant-derived compounds influence microbial metabolism and host physiology across ruminant species.

Data availability statement: All sequence data in this study was deposited in the NCBI Sequence Read Archive database under accession number: PRJNA1148777, http://www.ncbi.nlm.nih.gov/bioproject/1148777.

Funding: This research was funded by the Nagqu city major science and technology project (NQKJ-2023-10), the Department of Science and Technology of the Tibet Autonomous Region (XZ202101 ZD0002N), the breeding and efficient propagation of yaks in Gesangtang in Linzhou county (QYXTZX-LS2020-01), and the Ministry of Agriculture and Rural Affairs of China (CARS-37).

Acknowledgments: None.

Authors contributions: YZ and WB developed the study concept and design. YC, ZC, JT, and XZ conducted the experiment and collected the data. YC, MS, PP, YZ and WB analyzed the data and prepared the article. YZ and WB provided critical revisions to the manuscript. All authors have reviewed and approved the final version of the manuscript for publication.

Declaration of competing interest: No potential conflict of interest was reported by the author(s).

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